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Comparative Biodistribution of meta-Tetra(Hydroxyphenyl) Chlorin in Multiple Species: Clinical Implications for Photodynamic Therapy

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Background and Objective: **To optimize photodynamic therapy, it is necessary to know the distribution of photosensitizer in normal tissue as well as tumors and to know how well animal models match human. This study measured the biodistribution of meta-Tetra(Hydroxyphenyl) Chlorin (mTHPC) in three species of animals and in humans.**
Study Design/Materials and Methods: **mTHPC was injected intravenously into dogs, rabbits, rats, and humans, and drug levels in various tissues were determined 6 days later. One dog was perfused with 3 L of saline to remove blood trapped within organs.**
Results: **Absolute and relative concentrations of drug in specific tissues varied between species and between individuals. There was a general pattern of distribution. Highly vascularized tissues had the highest levels of mTHPC, not simply due to trapping of blood. mTHPC did not localize in bone and did not cross the blood-brain barrier. Humans had much higher levels of drug in their plasma and tissues than did animals.**
Conclusions: **First, drug retention varies from one tissue to another. Second, there is significant variability from one individual to another, whether animal or human. Third, current models cannot accurately predict from animal studies the optimum dose for humans. Measurement of photosensitizer level in plasma at time of treatment would allow optimal photodynamic dosing. Lasers Surg. Med. 20:437–442, 1997.**

Key words: human; mTHPC; PDT; pharmacokinetics; photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) has the potential to be a powerful treatment modality for both benign and malignant tumors, either as a primary therapy or adjunctive treatment. The therapy is based on a two-component modality consisting of a photosensitizing drug that ideally would localize selectively in the target tumor and light that activates the photosensitizer, causing cell death [1]. Alone, neither component would damage the tumor or normal tissue. Together, if the selectivity of the drug were sufficient and the light appropriately directed and optimally dosed, the tumor would be destroyed with minimal involvement of the adjacent normal tissue. In the clinical setting, the photosensitizer is injected in-

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travenously or intralesionally, or applied topically. A given delay time is chosen, specific to the drug and the target tissue, which should permit selective uptake or retention, and light activation is then applied [2,3].

Although the foundations of this therapy have been known for many years [1], practical use of PDT is just now beginning to meet its potential. There are several reasons why this might be so. A great deal of research has been responsible for development of improved light sources and novel photosensitizers, resulting in commercially available high power and compact lasers and drugs with shorter half-lives as well as higher tumor to normal tissue selectivity. One of the major problems with early photosensitizers was minimal selectivity (due, in part, to inappropriate drug dose) and prolonged photosensitivity [4,5]. Because of this, suboptimal dosing was often used to minimize damage to normal tissue with reduced therapeutic results, or alternatively high doses were used that caused complications with normal tissue. To be effective, and yet safe, it is necessary to optimize both the drug and light doses. Parameters that must be considered include the pharmacokinetics of the drug in the plasma, its distribution in normal tissues of different organs, the propensity for a given type of tumor to retain the drug, the interval between drug administration and light exposure that would optimize specific localization in tumor tissue compared to surrounding tissue, and effective light dose that can be safely applied.

Our laboratory studies over the past 4 years have focused on developing an animal model that would permit optimization of these parameters. This model allows evaluation of treatment protocol and full tissue analysis methodology, with rapid and reliable evaluation of any novel sensitizer [3,6–10]. We have worked extensively with the second-generation photosensitizer meta(tetrahydroxyphenyl) chlorin (mTHPC) [3,6,7,9,11–12], a "hard drug" that is not metabolized but is effectively and nearly completely eliminated via the liver. This type of drug provides significant advantages for biodistribution studies over drugs that are metabolized, since it is a stable and well-documented single molecular species with defined spectroscopy.

In this study we have compared the biodistribution of mTHPC in normal tissues of three animal species and limited tissue analysis of human. We have found marked differences in concentrations in the various tissues, differences between species, and differences between the animals and humans. We conclude that animal studies are approximations that can be only partially used to predict appropriate drug dose for clinical studies.

MATERIALS AND METHODS

Photosensitizer

The m-THPC used in this study, batch pK23K, was obtained from Scotia Pharmaceuticals (Guildford, Surrey, UK). The formulation was composed of 91.7% m-THPC, 5.9% meso-tetra(hydroxyphenyl)porphyrin, and 2.4% hydroxylated derivatives of m-THPC. The drug, supplied as a sterile lyophilized powder, was dissolved in a mixture of polyethylene glycol 400 and ethanol to a final concentration of 4 mg/ml and injected at a dose of 0.3 mg/kg body weight.

Animals and Patients, Drug Injections, and Tissue Sampling

Institutional guidelines regarding animal experimentation were followed. The study had Institutional Animal Care Committee approval. Three species of animals were used for this study. Dutch-belted rabbits had a mean body weight of 2.5 kg. Animals were anesthetized with 1–1.5 ml intramuscular ketamine hydrochloride (Ketaset, Aveco)/xylazine hydrochloride (Rompun, Mobay Corp). mTHPC was administered intravenously via ear vein and the line flushed with saline. Mongrel dogs weighed between 7 and 10 kg. They were anesthetized with intramuscular ketamine/rompun (12.5/7 mg/kg), and mTHPC was administered via leg vein infusion. Nude rats weighed between 200 and 400 g. They were sedated with intraperitoneal pentobarbital (40 mg/kg) and mTHPC was administered via catheter placed in the tail vein. After 6 days (7 days for dogs) the animals were euthanized, blood taken for drug level in plasma, organs harvested and washed free of excess surface blood. Total organ weight was determined. Organs were then divided into smaller tissue samples of 10–20 mg. Samples were weighed, placed in cryotubes, and frozen at −70°C until used for extraction. Plasma samples from additional rabbits injected with 0.3 mg/kg mTHPC were taken at time of previous PDT studies [3].

One patient, suffering from mesothelioma, was injected with 0.3 mg/kg mTHPC. Six days later, the chest cavity was opened, PDT done, and samples of lung, skin, and plasma taken. The pa-

tient died 5 days later of adult respiratory distress syndrome, and biopsies of additional limited organs were taken as part of postmortem analysis. These tissues were immediately frozen on dry ice, shipped to our laboratory, and weighed at time of analysis. Plasma samples were also available from other patients at time of endoscopic PDT for laryngeal tumors, a phase I/II clinical study taking place at LIJ for the past 2 years. These patients were injected with lower concentrations of mTHPC, ranging from 0.0375 mg/kg to 0.15 mg/kg and their plasma pharmacokinetics followed daily [9,13].

Drug Concentration Measurements

Blood was centrifuged at 2,000 r.p.m. for 10 minutes and plasma removed for direct spectrofluorometry. Tissues were frozen in liquid nitrogen and pulverized in a teflon chamber with a steel ball, using a Braun Micro Dismembrator II. One ml of dimethyl sulfoxide was added to every 10 mg of tissue, rotated on a wheel for 1 hour, and the suspension centrifuged at 15,000 RPM. The plasma or supernatant from tissue extraction was excited at 420 nm in a Shimadzu RF-540 spectrofluorophotometer with an emission range of 600–700 nm. The height of the 652 nm peak was compared to an m-THPC standard provided by Scotia Pharmaceuticals (Guilford, UK). This technique permitted an analysis of up to 40 samples per day. The lowest detectable concentration with a reliable signal to noise ratio was 40 pg/g tissue.

Perfusion Blood Trapping Study

One mongrel dog was injected with drug as described above. On the 6th day, the dog was anesthetized, placed supine, a midline incision was made from the sternum to the lower abdomen, and the thoracic aorta was isolated. A 12 Fr. catheter was inserted into the descending thoracic aorta and an incision was made in the inferior vena cava. Three liters of normal saline at 37°C were flushed under pressure into the aortic catheter in order to wash out the blood. Venous blood was suctioned and collected from the inferior vena cava. Efficiency was monitored by whitening of the liver. Postmortem, organs were harvested and analyzed as described above.

RESULTS

In order to determine whether the distribution of mTHPC was comparable in normal tissues from different species, we analyzed its concentration in multiple organs from dogs, rabbits, and rats at the postinjection time range that we previously determined optimal for PDT [3]. In addition, we had limited samples from human. mTHPC levels varied markedly between tissues and between species for the same tissue (Table 1). Absolute plasma levels also varied between species, ranging from 7 to 300 ng/ml. This is of special interest because we have shown previously that clinical response to PDT is correlated with the absolute plasma levels of photosensitizer [3,10]. Studies in humans given lower doses of mTHPC confirm that the plasma levels in humans are much higher than in the laboratory animals studied (data not shown) [9,13].

The variability seen between species was also reflected at the individual level, although the general distribution grouping did not change. This variability was much greater in some tissues than others, with lung, liver, kidney, and spleen

TABLE 1. Interspecies Biodistribution of mTHPC in Normal Tissues*

	Concentration of mTHPC (ng/g)			
Tissue	Dogs (5)	Rabbits (3)	Rats (2)	Human (1)
Kidney	487 ± 90	212 ± 217	340 ± 50	ND
Lung	277 ± 186	1,702 ± 1,786	470 ± 120	500
Heart	250 ± 24	159 ± 91	285 ± 45	100
Sm. intestine	235 ± 55	314 ± 190	85 ± 5	ND
Liver	226 ± 114	340 ± 188	220 ± 3	700
Diaphragm	162 ± 53	313 ± 210	195 ± 5	500
Stomach	148 ± 33	359 ± 350	155 ± 55	ND
Tongue	143 ± 25	406 ± 280	185 ± 25	ND
Spleen	142 ± 91	619 ± 481	360 ± 120	ND
Bladder	140 ± 31	320 ± 220	165 ± 25	ND
Esophagus	122 ± 38	226 ± 148	126 ± 26	ND
Trachea	120 ± 14	148 ± 64	105 ± 15	ND
Aorta	105 ± 23	355 ± 115[a]	120 ± 20	200
Skin	73 ± 10	62 ± 57	190 ± 30	100
Oral mucosa	72 ± 23	220 ± 155	ND	400
Sk. muscle	65 ± 32	66 ± 42	ND	100
Plasma[b]	17 ± 6	8 ± 5[c]	7 ± 4[a]	300
Bone	0	0	0	ND
Brain	0	0	0	ND

*All animal studies were done with 0.3 mg/kg mTHPC, injected 6–7 days prior to assay. Dog plasma levels were measured on day 6, other tissues on day 7. Human tissue samples, with the exception of lung, skin and plasma, were analyzed 11 days after injection of drug. Tissues are rank ordered for dog, which had the largest sample number. Numbers in parentheses following each species name indicate the number of individuals analyzed.
[a]Based on only two samples.
[b]ng/ml.
[c]Based on 16 rabbits.
ND = not determined.

TABLE 2. Relative Biodistribution of mTHPC in Normal Tissues

	Rank order of concentration of mTHPC			
Tissue	Dogs (5)	Rabbits (3)	Rats (2)	Human
Kidney	1	12	3	ND
Lung	2	1	1	2
Heart	3	13	4	6
Sm. intestine	4	8	14	ND
Liver	5	6	5	1
Diaphragm	6	9	6	2
Stomach	7	4	10	ND
Tongue	8	3	8	ND
Spleen	9	2	2	ND
Bladder	10	9	9	ND
Esophagus	11	10	11	ND
Trachea	12	14	13	ND
Aorta	13	5[a]	12	5
Skin	14	16	7	6
Oral mucosa	15	11	ND	3
Sk. muscle	16	15	ND	6
Plasma	17	17[b]	15	4
Bone	18	18	16	ND
Brain	18	18	16	ND

*Number of individuals assayed are indicated by numbers in parenthesis following each species name. Tissues are rank ordered for dog, which had the largest sample number. Two tissues with the same rank order indicate equivalent concentrations. The low values for human reflect the limited number of tissues analyzed.
[a]Based on two samples.
[b]Based on 16 rabbits.
ND = not determined.

TABLE 3. Relationship Between Size, Surface Area, and mTHPC Plasma Content*

Species	Av. Weight (kg)	Surface area (m^2)	Plasma content (ng/ml)	Ratio plasma/ surface area dose
Dog	7.5	0.65	22	6.5
Rabbit	2.2	0.14	12	2.5
Rat	0.15	0.025	7	3.9
Human	60	1.5	300	25.0

*0.3 mg/kg mTHPC was injected and plasma concentrations determined 6 days later. Note that since the total dose of drug was based on body mass, if the plasma concentration of all species was determined simply by body mass, all species would have the same plasma levels. We also calculated dose per m^2 and determined the ratio of plasma concentration to that dose. Note the variability between species and the high ratio in humans.

especially variable. Plasma levels also varied from one individual animal to another. We have seen variations of threefold in plasma between different rabbits [3].

The comparative distribution of mTHPC is shown in Table 2 to facilitate comparisons between tissues. Relative distribution of mTHPC varied from one species to another. For example, the organs with the highest levels were kidney and lung in dogs, and lung and spleen in rabbits. Although not all of the organs were available from humans, lung and liver had very high levels of drug. Mucosal levels (trachea and oral mucosa) was higher in rabbits and humans than skin, but the skin of the nude rats was higher than either mucosa or muscle. Despite these differences, there was a general pattern of distribution that was maintained across species. Highly perfused organs generally had the highest levels of mTHPC. Bone and brain had the lowest levels, with plasma low in all the animals except humans.

We considered the possibility that elevated concentration in highly vascularized tissues was a reflection of blood trapped within the organ. To test this, we perfused a dog with large volumes of saline just before sacrifice to remove the blood from organs such as the liver. At the end of the perfusion, the liver appeared white. In spite of this, the levels of mTHPC in the perfused tissues were comparable to nonperfused tissues. For example, the two perfused kidneys had a mean concentration of 340 ng/g, within the range difference from one individual animal to another. Even more striking, the perfused liver had a concentration of 370 ng/g and unperfused livers had a mean value of 226 ng/g. Therefore, highly vascularized organs have elevated photosensitizer levels within the tissue matrix or within the cells, not just within trapped blood.

The standard photosensitizer dosing protocols for PDT are based on body weight or surface area. From this, it is generally assumed that these parameters would predict plasma levels of photosensitizer. However, this is not the case (Table 3). If the achieved plasma level at time of treatment were dependent on body size, all species would have had comparable plasma levels at 6 days. We also calculated the dose per square meter of surface to determine whether the plasma level would correlate with that value. Again, the three species of animals vary, and humans are much higher. We have measured the clearance and found that the half-life in rabbits is 24.7 hours [3], compared to 44.5 hours for humans [9,13], consistent with this data.

DISCUSSION

We have analyzed the distribution of mTHPC in multiple organs of different species at 6–7 days postinjection, our preferred interval for PDT based on preferential retention in target tissues with reduced nonspecific plasma content [3,9,13]. We are unable to compare these results with distribution of other photosensitizers, including Photofrin™, which is the only PDT drug fully approved in the United States. We have no data for other drugs using our extraction process, except for partial data for protoporphyrin IX synthesized in vivo from systemically administered δ-amino levulinic acid. A major advantage of using a drug such as mTHPC for the studies described here is the absence of metabolic alteration by the peripheral tissues. Therefore, we can look simply at distribution of photosensitizer, rather than dealing with a complex pattern combining distribution with synthesis or destruction of the molecule of interest.

A similar overall pattern of organ distribution of mTHPC was true for all three animal species and for humans. Levels of drug can be broken down into three groups: high, moderate, and low concentrations. Highly perfused organs such as lung generally had the highest concentration of drug. Lowest levels were found in plasma, bone, and brain. The absence from brain in all three animal species suggests that mTHPC does not cross the blood-brain barrier. Other tissues, including skeletal muscle and most mucosa, tended to be intermediate.

Korbelik and Krosl [14] recently reported that the direct killing of tumor cells by PDT using Photofrin™ was dependent on proximity to the blood supply. Those cells closer to the blood supply were more likely to be destroyed than those farthest away, highlighting the importance of blood supply in delivering photosensitizer to the target tissue. We have shown that the elevated concentration in highly vascularized tissues is not simply a reflection of blood trapped within the organ. High capillary density appears to alter the absolute retention of drug in the tissue. This must be carefully considered if PDT is planned for tumors in highly vascularized organs such as liver or spleen.

We found variability in mTHPC concentration between species and between individuals within a single species. This variability reflects, in part, variability within a single organ. For some tissues (e.g., muscle such as tongue that is covered by mucosa, the levels are different in the two tissue compartments. Therefore, two biopsies of the same organ can differ, depending on the exact site of the biopsy. In other tissues, such as kidney, the extent of perfusion in different parts of the organ could alter the drug levels. However, the intraspecies variability was greater than the variability within a single organ. The individual variability can also be systemwide, with all tissue levels elevated. For this reason, ranking of levels in tissues (Table 2) facilitates comparison.

The highest plasma level seen, 300 ng/ml, was in a single patient given 0.3 mg/kg mTHPC. Fifteen patients given 0.15 mg/kg in a phase II clinical trial conducted jointly at LIJ and Orebro Medical Center had a mean plasma level of 195 ± 105, confirming that this high level was not an aberration of the one patient [9,13]. In contrast, plasma levels in the animals injected with 0.3 mg/kg ranged from 4 to 21 ng/ml in rabbits, and 12 to 27 ng/ml in dogs. Therefore, humans show the highest levels of drug in plasma, whereas all species appear to show marked variability in plasma level from one individual to another.

Three major conclusions can be drawn from this study. First, it is not possible accurately to predict from animal studies the optimum dose to be given to humans in clinical trials. Second, it is not possible to predict from a small sample of normal tissues what the drug retention will be in other tissues. This means that the photosensitivity of normal tissue adjacent to a tumor can vary widely, depending on the tissue. Finally, there is significant variability from one individual to another, whether animal or human.

From these observations one might conclude that proper predictive dosing for PDT is not possible given the conventional approaches of using body weight or surface area. We have argued previously [8–10] that the level of photosensitizer in plasma and tissue is a complex function of the species' weight, surface area, total blood volume, liver size and efficiency, and capillary density. At present, our modelling capabilities are insufficient to provide accurate predictions of the plasma levels at the time of treatment. But we have shown previously that clinical response to two different photosensitizers in our animal tumor model correlated with plasma level at time of treatment [3,8,10]. We therefore propose that individual tailoring of light dose, based on patient's plasma level of photosensitizer at time of therapy, can compensate for this unpredictability. This approach should have general applicability and

therefore be useful for other photosensitizers besides mTHPC, including Photofrin™. We have been following this approach in our phase I/II trials at both hospital centers. In 45 treatments delivered to 33 patients, plasma content at time of treatment has always been measured and photodynamic light dose adjusted accordingly.

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